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Research Article

MICROBIOLOGICAL QUALITY OF LETTUCE (*Lactuca sativa*) CONSUMED ON THE STREETS MAROUA (CAMEROON): EFFECT OF DISINFECTING AGENTS USED BY SOME VENDORS

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Abstract- This study was carried out to determine and compare the microbiological quality of lettuce (*Lactuca sativa*) from market (unwashed) and those ready to eat in Maroua. The results revealed that 92.3% of lettuce vendors in Maroua, used Chlorine and 7.7% used potassium permanganate as cleaning method. The microbial concentration of lettuce varied from one vendor to another. Total flora ranged from 2.3 to 4.9 Log CFU/g for unwashed samples and from 2 to 4.51 Log CFU/g for washed samples; fungi counts were comprised between 0.76 to 2.65 Log CFU/g for unwashed samples and 0.26 to 2.17 Log CFU/g for washed samples; total coliforms ranged from 2.84 to 3.60 Log CFU/g for unwashed, and 0.26 to 3.00 Log CFU/g for washed lettuce; fecal coliforms ranged from 1.29 to 3.60 Log CFU/g for unwashed samples and 1.70 to 3Log CFU/g for washed samples. These results show also that *Vibrio spp.* was found amongst the 69% of unwashed samples, but was absent in all the washed samples. *Salmonella spp.* was present in 85% samples of unwashed lettuce, but absent in washed samples. The results revealed also that; there was a significant difference between the bacterial concentration of unwashed and washed lettuce samples ($P<0.05$). Most of the analysed samples (92%) composed of washed and unwashed samples were not satisfactory for consumption.

Key words: Microbiological Quality, Washed Lettuce, Unwashed Lettuce, Disinfectant.

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Introduction

Vegetable is an edible part of a plant having a salty or sour taste but not sweet, intended for cooking or eating raw [1]. The role of fresh fruits and vegetables in nutrition and healthy diets is well recognized and in recent years, many countries have undertaken initiatives to encourage consumers to eat more of these products [2,3]. The increasing consumption of raw foods and vegetable origin gains importance, as they are an important source of vitamins, fibers and minerals [4]. Antioxidants content of vegetables, is particularly thought to be able to protect human cells from the attack of free radicals, which is in turn involved in the etiopathogenesis of most chronic diseases [5]. Among these vegetables, there is lettuce, which has very low calorie content and is composed primarily of water, about 90 to 95%. The lettuce also contains fibers; substances like minerals, potassium, calcium, phosphorus, iron and magnesium and antioxidants such as beta carotene and vitamins A, C and E, vitamin K, foliate as well as many vitamin B complex [6]. For many countries, particularly developing countries, such products have become valuable, making a substantial contribution to the economy as well as to the health of a country's population [7]. However, recent food safety problems such as food poisoning, food spoilage, food contamination, mishandling of food were linked to these products [8-12]. For nutritional health and economic reasons, it is important that the consumption of fresh products continues to increase. Therefore, efforts at the international level to resolve food safety problems linked to fresh products are essential and timely [7]. The Far-North region of Cameroun has registered in this last decade epidemic like cholera and diarrhea, probably due to the consumption of poorly handled foods, especially those sold around the streets due to the increasing urbanization, creation of a university and other institutes which has led to the multiplication of fast-foods. But there are not yet data on diseases associated with the consumption of lettuce in Maroua. Amongst these foods, there is lettuce, which is sometimes accompanied by other items such as onions, tomatoes, cucumbers and pear and, can also be served with roasted fish, meat, etc. Since lettuce is eaten raw, if it is not well handled, it can lead to a number of illnesses, it will be important to know its microbiological quality. Meanwhile, their harvesting, distribution, and commercialization brought to light a microbiological risk associated with these products [13-15]. Lettuce is a greenish vegetable widely consumed in the Far North region of Cameroon. It has very high nutritive content; "sedative" and "painkilling" properties, which make it an effective juice for treating sleep anxiety and nervous disorders [16]. Despite the numerous advantages, it is essential to determine the microbiological quality of this leafy vegetable, before and when it is ready to eat. Another objective of this study is to determine the effect of disinfecting agents used by the vendors.

Materials and Methods

Investigation and Sampling

An investigation was conducted amongst the 13 vendors of lettuce in order to know the type and quantity of disinfectant used when the samples were washed. February to April 2015, seventy height lettuce samples were collected from 13 vendors (6 samples for each) of five different quarters: Comice, Domayo, Ouro-tchede, Djarengol and Harde in Maroua [Table-1]. The samples analyzed included unwashed and washed lettuce. The unwashed lettuce samples were those boughs from market by the vendors, while the washed or ready to eat lettuce were collected after washing and disinfection. Each vendor was sampled 3 times and the analysis was performed in duplicate. The samples of lettuce were collected by using sterile gloves and placed in sterilized polyethylene bags, transported to the laboratory in ice chests and processed on the same day.

Table-1 *numbers of unweshed and washed samples collected*

Samples of lettuce	Location	Numbers of unwashed samples	Numbers of washed samples	Total
1	Djarengol 1	3	3	6
2	Djarengol 2	3	3	6
3	Comice	3	3	6
4	Ouro-tchede 1	3	3	6
5	Ouro-tchede 2	3	3	6
6	Domayo 1	3	3	6
7	Domayo 2	3	3	6
8	Domayo 3	3	3	6
9	Domayo 4	3	3	6
10	Domayo 5	3	3	6
11	Harde 1	3	3	6
12	Harde 2	3	3	6
13	Harde 3	3	3	6
Total		**39**	**39**	**78**

Preparation for microbial analysis

Each sample of unwashed and washed lettuce (25g) were weighed aseptically into sterile stomacher bags, diluted with 225 mL buffered peptone water (Oxoid Cambridge, UK) and homogenized using a stomacher for 2 min [17,18]. From this mixture obtained, decimal serial dilutions were done subsequently.

The homogenates obtained from the samples preparation were used for different plating and incubation procedures. A volume of 100µL of each diluted sample was plated in duplicate. For aerobic mesophlic plate count, the samples were spread on Plate Count Agar (Oxoid, Basingstoke, UK) and incubated at 30°C for 72 h [19]. Colonies between 30-300 were counted and expressed as Colony forming units (CFU)/g of lettuce. Presumptive test for coliform bacteria was done; 100µL of the diluted samples was then spread on sterilized and solidified Violet Red Bile Agar (VRBA; Oxoid, Scharleau, Espagne) and incubated at 37°C for total coliforms and 44°C for fecal coliforms. After 24 hours, purple-red colonies that were 0.5 mm and surrounded by zone of precipitated bile acids were counted. For confirmation, selected colonies were transferred to a tube of Brilliant green lactose bile (BGLB) broth 2% (total coliforms) or Lauryl Tryptose broth (Fecal coliforms), incubated at 35°C and examinated after 24 and 48h for gas production [17]. Fungi (yeasts and molds) were isolated by inoculating 100µL of the samples on Sabouraud Dextrose agar (Fluka, Sigma-Aldrich, India) with Chloramphenicol. After incubation at 25°C for 3 to 5 days, the colonies obtained were then counted [20]. For Salmonella detection, broth enrichment technique was used [21]. First the stomached mixture lettuce-buffered peptone water (25g in 225mL) was incubated for 18 to 24 h at 37°C. One milliliter was added to 100 ml of Rappaport-Vassiliadis broth (Merck, Germany) and Muller Kauffmann broth at (37°C for 24h) for selective enrichment. A loop-full of enriched broth was then plated on the XLD agar (Oxoid, Basingstoke, England) and incubated at 37 °C for 24 h. Suspect colonies or those with black center were streaked on nutrient agar (Oxoid) and confirmed by following biochemical tests: triple sugar/iron test, urea test, L-lysine decarboxylation test, β-galactosidase test, Voges-Proskauer test and indole test. Confirmation was also carried out using API 20E (Biomerieux, France).

For *Vibrio* count, enrichment was first performed by adding 1mL of the homogenized sample solution to alkaline peptone water and incubated at 37°C for 6 h. The enriched sample was then streaked on thiosulfate-citrate-bile-sucrose (TCBS; Merck, Germany) agar plates and incubated at 37°C for 18 to 24 h. Yellow and green colonies were subjected to Grams' stained [22], salt tolerance and sugar fermentation (glucose, sucrose, lactose and arabinose), catalase activity, motility and indole [23-26].

Statistical analysis

The variance of the different repetitions was obtained by using Microsoft Office Excel 2007 software. On Statgraphics Centurion 17.1.06. Software, ANOVA with one factor, followed by The Duncan test was performed to compare the different unwashed and washed samples of lettuce from each site.

Results

Investigations about disinfectant used revealed that, 92.3% of vendors in Maroua, used Chlorine and 7.7% used potassium permanganate in order to reduce the microbial load in lettuce. Among these vendors, 46.2% used 9mL of chlorine (8°) in 5L of water, 7.7% used 9mL of chlorine (8°) in 1L of water, 15.45% used 9ml of chlorine (8°) in 10L of water, 7.7% used 9mL of Permanganate in 10L of water, 7.7% used 75mL chlorine (8°) in 20L of water, 7.7% used 18mL of chlorine (8°) in 5L of water and 7.7% used 9mL of chlorine (8°) in 6L of water.

Total and fungi flora counts of different samples

The microorganism's concentration of the samples examined varied in function of different vendors from the same location as well as for different locations [Table-2]. Aerobic plate counts ranged from 2.3 Log CFU/g for sample 4 (Ouro-tchédé 1) to 4.99 Log CFU/g for sample 2 (Djarengol 2) for unwashed lettuce samples; while for washed samples, it was between 2 Log CFU/g for samples 4, 5 (Ouro-tchédé 1 and 2) and 4.51 Log CFU/g for sample 7 (Domayo 2). The lettuce samples values from all the locations were lower than this value and also respected the Canadian reference range (total mesophilic aerobic bacteria<8 Log CFU/g). This norm was used because there is no legislation that includes microbiological criteria for raw vegetables in Cameroon.

Table-2 *Total and Fungi flora of different washed and unwashed lettuce samples*

Location sites		Total flora (LogCFU/g)		Fongi flora (LogCFU/g)	
		Unwashed	Washed	Unwashed	Washed
S1 :	Djarengol 1	4.90±0.01i	3.36±0.03d	1.45±0.14ef	1,36±0.08fg
S2 :	Djarengol 2	4.99±0.09j	3.48±0.00e	1.69±0.12gh	0.76±0.15d
S3 :	Comice	3.29±0.11c	2.90±0.01c	0.76±0.01a	0.26±0.02b
S4 :	Ouro-tchede 1	2.3±0.04a	2.00±0.03a	0.99±0.09b	0.48±0.00c
S5 :	Ouro-tchede 2	3.00±0.01b	2.00±0.07a	1.78±0.04h	1.43±0.03g
S6 :	Domayo 1	4.58±0.02g	4.48±0.01k	1.56±0.08gh	1.15±0.07e
S7 :	Domayo 2	4.72±0.02h	4.51±0.05k	1.40±0.03de	1.19±0.14ef
S8 :	Domayo 3	4.45±0.03f	4.18±0.02j	1.11±0.10bc	NDa
S9 :	Domayo 4	3.93±0.02c	3.60±0.04g	2.00±0.01i	0.77±0.03d
S10 :	Domayo 5	4.70±0.03h	4.01±0.03i	2.65±0.05j	2.17±0.00h
S11 :	Harde 1	3.47±0.07d	2.70±0.03b	1.27±0.09d	1.08±0.07e
S12 :	Harde 2	4.70±0.03h	3.56±0.02f	1.56±0.04fg	1.43±0.01g
S13 :	Harde 3	4.63±0.01gh	3.81±0.05h	1.25±0.07cd	1.04±0.04e

The values of the same location, follow by different letters are significantly different ($p<0.05$); Nd: not detected

The fungi flora concentration of unwashed lettuce was comprised between 0.76 Log CFU/g for the sample 3 (Comice) and 2.65 Log CFU/g for the sample 10 (Domayo); while that of washed lettuce was comprised between 0.26 Log CFU/g for the sample 3 (Comice) and 2.17 Log CFU/g for the sample 10 (Domayo 5). The lettuce samples from all the locations respected the Canadian reference range for molds and yeast (<10^5CFU/g). In Cameroon there is not yet legislation that includes microbiological criteria for raw vegetables.

Total and fecal coliforms, *Vibrio* and *Salmonella spp* of different unwashed and washed lettuce samples

The numeration of total coliforms, fecal coliforms, *Vibrio*, *Salmonella* was carried out. The results obtained from the different washed and unwashed samples of lettuce, presented in [Table-3], revealed that the microbial load of lettuce varies in function of vendors from the same as well as from different location. For total coliforms, the values of unwashed lettuce were comprised between 2.84 for the sample 12 (Harde 2) and 3.60 Log CFU/g for the sample of Djarengol 2. Those of washed lettuce range between 0.26 for the sample 12 (Hardé 2) and 3.00 Log CFU/g for the samples 1; 8; 11 (Djarengol; Domayo 3 and Hardé 1). The washed and unwashed lettuce samples in all the locations did not respected the Canadian reference range (total coliforms <10^2CFU/g) except sample 12. The concentration of fecal coliforms in unwashed samples varies between 1.29 for S12 (Harde 2) and 3.60 Log CFU/g for S1 (Djarengol 2) while for washed lettuce the value is between 1.70 for S6 (Domayo 1) and 3 Log CFU/g for S1, S2 and S10 respectively Djarengol (1 and 2) and Domayo 5. All unwashed lettuce samples did not respect the Canadian reference range (total coliforms < 10 CFU/g), except washed sample 12 from Hardé 2. These results show also that *Vibrio* was found in 9 samples of unwashed lettuce while they were absent in all the 13 washed samples. *Salmonella spp* were present in 11 samples of unwashed lettuce but, absent on washed samples. Analysis of variance was carried out on bacterial/fungi colonies obtained from each unwashed and washed lettuce sample. The results revealed that; there was a significant difference between unwashed lettuces and washed lettuce with disinfectant ($P<0.05$) for total coliforms, fecal coliforms, total flora. For the fungi flora, there was no significant difference between unwashed, and washed lettuce sample for sample 1 and 12 ($P>0.05$).

Table-3 Total *and fecal coliforms, Vibrio spp., Salmonella spp. counts of washed and unwashed lettuce samples*

Location sites		Total coliforms (LogCFU/g)		Fecal coliforms (LogCFU/g)		*Vibrio*		*Salmonella*	
		Unwashed	Washed	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
S1 :	Djarengol 1	3.50±0.07^{g}	3.00 ±0.07^{e}	3.22±0.04^{e}	3.00±0.04^{f}	+	-	+	-
S2 :	Djarengol 2	3.60±0.05^{h}	2.07± 0.04^{b}	3.60±0.01^{h}	3.00±0.00^{f}	+	-	+	-
S3 :	Comice	3.08 ±0.07^{c}	2.60±0.03^{d}	3.08±0.02^{d}	2.28±0.03^{c}	+	-	+	-
S4 :	Ouro tchede 1	2.99± 0.09^{bc}	2.04± 0.04^{b}	3.00±0.04^{c}	2.90±0.01^{de}	+	-	+	-
S5 :	Ouro tchede 2	3.27 ± 0.03^{ef}	2.90± 0.00^{e}	3.18±0.01^{e}	3.00±0.02^{f}	+	-	+	-
S6 :	Domayo 1	3.22 ± 0.04^{de}	2.30±0.09^{c}	3.18±0.04^{e}	1.70±0.04^{b}	+	-	+	-
S7 :	Domayo 2	3.28 ± 0.05^{e}	2.70± 0.02^{ef}	2.95±0.03^{bc}	2.84±0.02^{e}	+	-	+	-
S8 :	Domayo 3	3.17± 0.06^{d}	3.00 ±0.07^{e}	3.15±0.00^{e}	2.99±0.08^{ef}	+	-	+	-
S9 :	Domayo 4	3.30± 0.01^{f}	2.97± 0.01^{e}	3.29±0.01^{f}	2.91±0.03^{de}	+	-	+	-
S10 :	Domayo 5	3.30± 0.00^{f}	2.97± 0.01^{e}	3.30±0.03^{f}	3.00±0.05^{f}	+	-	+	-
S11 :	Harde 1	3.30±0.04^{e}	3.00± 0.1^{ef}	3.29±0.03^{f}	2.93±0.04^{def}	+	-	+	-
S12 :	Harde 2	2.84± 0.03^{a}	0.26± 0.02^{a}	1.29±0.09^{a}	Nd ^{a}	-	-	-	-
S13 :	Harde 3	2.93± 0.03^{b}	2.38±0.02^{c}	2.91±0.01^{b}	2.69±0.30^{d}	-	-	-	-

The values of the same location, follow by different letters are significantly different ($p<0.05$); +: presence; - : absence; nd: not detected

General quality and impact of disinfecting lettuce samples

[Table-4] presents the general impact of disinfection of different samples. These values are situated between 33.55% (Domayo 3) and 99.72% (Harde 2) for total coliforms; 19.33% (Ouro-tchede 1) and 96.66% (Domayo 1) for fecal coliforms; 18.21% (Djarengol 1) and 92.02% (Domayo 4) for molds and yeast; 20.81% (Domayo 1) and 97.1% (Djarengol 1) for total flora. In general, some impacts of disinfecting lettuce are more than 50%. The most important reduction of microorganisms by disinfecting agents has been observed on total coliforms with a rate of 84% over all the sampling sites. As a summary of this study only 2 samples of ready to eat lettuce were good for consumption, according to the norm [Table-5] : Sample 6 from Domayo 1 and sample 12 from Harde.

Table-4 *General impact of disinfecting lettuce samples*

Samples of lettuce		Total coliforms (%)	Fecal coliforms (%)	Fongi flora (%)	Total flora (%)
1	Djarengol 1	68.55±0.03	39.43±0.55	18.21±5.16	97.1±0.29
2	Djarengol 2	97.07±0.07	74.99±0.37	88.16±0.95	96.9±0.62
3	Comice	66.32±3.16	84.18±0.59	68.06±6.36	58.3±8.24
4	Ouro-tchede 1	88.83±1.26	19.33±4.12	69.17±6.29	49.73±4.04
5	Ouro-tchede 2	56.68±3.67	33.21±5.56	54.98±0.42	90±0.3
6	Domayo 1	87.96±1.34	96.66±0.07	61.58±4.64	20.81±6.80
7	Domayo 2	73.58±1.47	21.67±1.57	36.8±4.99	38.45±0.44
8	Domayo 3	33.55±2.49	31.21±0.93	92.02±1.89	46.33±2.05
9	Domayo 4	53.65±0.19	58.72±0.22	94.02±2.15	52.98±5.33
10	Domayo 5	53.85±0.76	49.86±2.76	66.63±3.50	79.39±0.22
11	Harde 1	49.8±3.03	56.4±0.22	36.44±2.92	83.03±2.76
12	Harde 2	99.72±0.12	95.67±4.04	24.49±9.10	92.74±0.93
13	Harde 3	71.65±2.85	40.6±3.13	38.36±4.78	85.12±0.00

Table-5 *General quality of different samples*

Samples		Criteria	Interpretation
1	Djarengol 1	Unwashed	Unsatisfactory
		Washed	Unsatisfactory
2	Djarengol 2	Unwashed	Unsatisfactory
		Washed	Unsatisfactory
3	Comice	Unwashed	Unsatisfactory
		Washed	Unsatisfactory
4	Ouro tchede 1	Unwashed	Unsatisfactory
		Washed	Unsatisfactory
5	Ouro tchede 2	Unwashed	Unsatisfactory
		Washed	Unsatisfactory
6	Domayo 1	Unwashed	Unsatisfactory
		Washed	Satisfactory
7	Domayo 2	Unwashed	Unsatisfactory
		Washed	Unsatisfactory
8	Domayo 3	Unwashed	Unsatisfactory
		Washed	Unsatisfactory
9	Domayo 4	Unwashed	Unsatisfactory
		Washed	Unsatisfactory
10	Domayo 5	Unwashed	Unsatisfactory
		Washed	Unsatisfactory
11	Harde 1	Unwashed	Unsatisfactory
		Washed	Unsatisfactory
12	Harde 2	Unwashed	Satisfactory
		Washed	Satisfactory
13	Harde 3	Unwashed	Unsatisfactory
		Washed	Unsatisfactory

Discussion

The fact that the vendor use chlorination as disinfecting method is not strange [27]. The most common method used to reduce microbial load of fruits and vegetables is the disinfection of washing water by chlorination [28], [29] Recent studies by Adjrah *et al.* [30] showed that another precaution to limit the risk of infection coming from vegetable is to wash them with potassium permanganate. Chlorinated water and potassium permanganate solution are mostly used to reduce microorganisms in lettuce and caused a reduction of almost 1 log in the number of aerobic mesophiles [31,32]. None of the vendors of ready to eat lettuce used more than one disinfectant in lettuce treatment. The efficacy of the method used to reduce microbial populations is usually dependent on the type of treatment; type an physiology of the target microorganisms, characteristics of produce surfaces, exposure time and concentration of cleaner/sanitizer, pH and temperature [32,33]. For some authors, the use of 100-150µg/mL of chlorine is sufficient to disinfect raw products like celery and lettuce [29].

The variation of total flora on the different unwashed and washed lettuce samples can be explained by the different ways that vendors handle lettuce and the environment in which the activity is carried out**.** These results correspond to those of Almeida *et al.* [27] on comparison of different washing and disinfection of lettuce in the town of Porto Alegre in Brazil. Our data are also consistent with the results of Cardamone *et al.* [31] who found that the aerobic mesophilic count level of fresh products on sale in Sicily (Italy) ranged between 2 log and 6 log CFU/g. These results showed that the total mesophilic aerobic bacteria for unwashed samples were lower. This could also be as results from the fact that most lettuce gardens in Maroua are watered with ground water of drilling.

Table-6 *ANOVA Table*

Microorganisms	Type of lettuce	Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Total coliform	Unwashed	Between groups	1,63982	12	0,1366	54,91	0,0000
		Within groups	0,0647102	26	0,0024		
		Total (Corr.)	1,70453	38			
	washed	Between groups	20,5691	12	1,7140	272,34	0,0000
		Within groups	0,16364	26	0,0062		
		Total (Corr.)	20,7328	38			
Fecal coliform	Unwashed	Between groups	11,0108	12	0,9175	559,38	0,0000
		Within groups	0,0426483	26	0,0016		
		Total (Corr.)	11,0534	38			
	washed	Between groups	24,8907	12	2,0742	733,44	0,0000
		Within groups	0,07353	26	0,0028		
		Total (Corr.)	0,00282808	38			
Fungi flora	Unwashed	Between groups	8,35096	12	0,6959	89,19	0,0000
		Within groups	0,202867	26	0,0078		
		Total (Corr.)	8,55383	38			
	washed	Between groups	11,6243	12	0,9686	90,68	0,0000
		Within groups	0,277738	26	0,0106		
		Total (Corr.)	11,9021	38			
Total flora	Unwashed	Between groups	26,0711	12	2,17259	891,19	0,0000
		Within groups	0,0633841	26	0,0024		
		Total (Corr.)	26,1345	38			
	washed	Between groups	24,7305	12	2,0608	4683,47	0,0000
		Within groups	0,0114408	26	0,0004		
		Total (Corr.)	24,7419	38			

This water is less contaminated with microorganisms. A maximum acceptable concentration of 5.0 log CFU/g for aerobic mesophiles is suggested by Mossel [3] and Solberg *et al.* [34]. Compared to the results obtained by Hagenmeaier and Baker [35] on bagged salad and those of Soriano et *al.* [36] obtained on the microbiological quality of lettuce served in university our samples of washed lettuce were less contaminated.

This study revealed a great reduction of the total flora after disinfection. Related studies carried out in Brazil by Almeida *et al*. [27] revealed that the greatest reductions in total mesophilic aerobic bacteria were found for the sodium hypochlorite (200 ppm of free chlorine) treatment for 30 min, with reductions of 2.46 log10 CFU/g and 2.35 log10 CFU/g, respectively. However, there were greater reductions in total flora according to Nascimento *et al*. [37] and Lopez-Galvez *et al*. [38] after disinfection with this product.

The presence of fungi flora on unwashed lettuce could be a consequence of their relationship with the environment (soil, air and water). Lettuce can be also be contaminated by humus, sprinkling water and manipulations as shown by Guiraud [39]. After disinfection, the presence of fungi flora could be either due to insufficient disinfection time, or to the lower concentration of chlorine or permanganate of potassium solution used in washing water.

The variation of total and faecal coliforms of the different unwashed lettuce samples can be explained in the same way as for total flora. All washed samples of lettuce with disinfectant did not respect the Canadian reference range ($>10^2$ CFU/g) [40,41], except samples 6 and 12 that fall under the Canadian reference ($\leq 10^2$). This could also be due to the variation of the quantity of disinfectant used by the vendors.

As for the total flora, the lower concentration of total coliforms could also be the

result of watering lettuce garden with ground water less contaminated compared with surface water [42]. The total coliforms could also be lower as a result of the fact that the storage time of the entire unwashed lettuce sample was low. According to Frank-Peterside and Waribor [43], bacterial load on leafy vegetables increase with time during storage. After disinfection of lettuce samples, there was a great reduction in total coliforms, showing the efficacy of disinfection. Mngoli and Ng'ong'ola-Manani [44] reported that total coliforms were within the reference range. It was shown that based on the fact that the microbial reductions obtained within 15 min and 30 min of exposure, there was no significant difference ($p<0.05$). The shorter the period of disinfection is an advantage, considering the hurry routines of food services.

Fecal coliforms are usually indicators of intestinal contaminants from man and animal. Since most unwashed samples are from the market, vendors spend the whole day at the market; the risk can build up during retail due to microbial proliferation. Increase in fecal coliforms contamination with time could be a result of unhygienic handling and use of refreshing water continuously during the day. Related studies carried out by Mngoli and Ng'ong'ola-Manani [44] demonstrated that fecal coliforms contamination increase with time. Many of the vendors of Maroua also use a single bucket of water the whole day to refresh products; this could also be a source of contamination as demonstrated the study of Amoah *et al*. [27]. Fecal coliforms were found on our samples of washed lettuce. These results correspond to those of Seo *et al*. [45] who reported that the fecal coliform population ranged from 2.2 to 7.5 log CFU/g. After disinfection with chlorine/permanganate, only 2 samples respected the Canadian reference range, thus making in general, sample 6 and 12 satisfactory to eat and the rest unsatisfactory because of high fecal coliforms concentration. The presence of these bacteria on washed lettuce could be the result of various manipulations of the vendors who use naked hands to serve clients the whole day. Coliforms are usually indicators of the probable presence of pathogenic organisms [46-48]. The presence of these bacteria after disinfection could be due to the fact that microorganisms can penetrate the lesion of vegetable tissues and remain inaccessible to the disinfectant [49-51]. As a result of this, in the bath, chlorine remains limited to the surface and microorganisms infecting tissues are not completely eliminated.

The presence of *Vibrio spp.* or *Salmonella* spp. on unwashed lettuce could be linked to the environment (soil, air and water) and natural fertilizers (animal dejections) in the farm. Some researchers demonstrated that *Vibrio cholerae* and six other species were detected on lettuce [52]. Moreover, many persons use farms like their toilet; for this reason, *Vibrio* and other Enterobacteria, present in the gastrointestinal tract of humans can easily be found on vegetables. After washing and disinfecting lettuce samples, there was a total absence of *Vibrio spp.* indicating that the bacteria were sensible and the concentrations of chlorine and permanganate of potassium used in washing water were sufficient. These results corroborate those carried out in Togo by Adjrah *et al.* [30] and which revealed no cases of *Vibrio* linked to the consumption of fresh cut vegetables.

These results show that some disinfected lettuce samples had high percentages of impact of disinfection indicating that the microbial load drops after disinfection. Reduction of microbial flora could be as a result of increase contact time with the disinfectant, clean working surfaces and utensils, the right proportion of disinfectant.

Conclusion

The general objective of this study was to determine and to compare the microbiological quality of the lettuce sold in the market (unwashed) and those consumed on the streets of Maroua (washed). The microbiological profile revealed that; the microbial load of the different samples varies in function of different vendors from the same location as well as different location. For total flora, total coliforms and fungi flora, the microbial load respected the norm. There was an exception to fecal coliforms in that; all unwashed samples did not respect the norm except sample twelve while all the washed samples did not respect the norm except sample six and twelve. *Vibrio spp., Salmonella spp.* were present the majority of unwashed lettuce samples, but totally absent in washed lettuce samples. Ready to eat lettuce was not well handled by vendors since most lettuce samples were contaminated with fecal coliforms except samples six and twelve. After disinfection by different vendors, only sample 6 from Domayo 1 and sample 12 from Harde 2, two samples of ready to eat lettuce sold in Maroua were satisfactory for consumption. For a country like Cameroon there is a need for specific regulations. It will be necessary to compare the effect and the right concentration of chlorine and potassium permanganate to disinfect lettuce.

Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Author Contributions

Maïworé J. conceived this study. But Anhyidong, Nkongho, Baane worked in the lab. Tatsadjieu performed statistical analysis, Mbofung revised the English version of the manuscript, all this on the guidance of Maïworé. The intervention of all the authors was notable during revision and interpretation of the results. All the authors read and approved the final manuscript.

Abbreviations

UK: United Kingdom
CFU Colony forming Unit
VRBA: Violet Red Bile Agar
BGLB: Brilliant green lactose bile
XLD: Xylose Lysine Deoxycholate
API: Analytical Profile Index
TCBS: Thiosulfate Citrate Bile Salts Sucrose
ANOVA: Analysis of Variance
S: Site
NSIF: National Social Insurance Fund
CIRAD : Centre de Coopération Internationale en Recherche Agronomique pour le Développement

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors

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